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EFFORT-RELATED MOTIVATIONAL DYSFUNCTIONS: BEHAVIORAL AND
NEUROCHEMICAL STUDIES OF THE WISTAR-KYOTO RAT MODEL OF
DEPRESSION

A Thesis Presented

by

BRENDAN E. ABBOTT

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

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Department of Neuroscience & Behavior

EFFORT-RELATED MOTIVATIONAL DYSFUNCTIONS: BEHAVIORAL AND
NEUROCHEMICAL STUDIES OF THE WISTAR-KYOTO RAT MODEL OF
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BRENDAN E. ABBOTT

Approved as to style and content by:

Mariana Pereira, Chair

David Moorman, Member

Jerrold Meyer, Member

Paul Katz,
Neuroscience & Behavior Graduate Program Leader

ABSTRACT

EFFORT-RELATED MOTIVATIONAL DYSFUNCTIONS: BEHAVIORAL AND NEUROCHEMICAL STUDIES OF THE WISTAR-KYOTO RAT MODEL OF DEPRESSION

MAY 2018

BRENDAN E. ABBOTT, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST
M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Mariana Pereira

Depression and related disorders are characterized by motivational dysfunctions, including deficits in behavioral activation and exertion of effort. Animal models of relevance to depression represent a critical starting point in elucidating the neurobiological mechanisms underlying motivational dysfunctions. The present study explored the use of the Wistar-Kyoto (WKY) animal model of depression to examine effort-related functions as measured by voluntary wheel running and performance on a mixed fixed ratio 5/progressive ratio (FR5/PR) operant task. Given the known link between motivational aspects of motivation and the mesocorticolimbic dopamine (DA) system, the behavioral effects of d-amphetamine (0.5 and 1.0 mg/kg, IP), a psychostimulant that increases DA release, were evaluated in WKY and control Sprague-Dawley (SD) male and female rats responding on a mixed FR5/PR task. An additional experiment assessed intracellular content of monoamine neurotransmitters and their metabolites in relevant mesocorticolimbic brain regions, including the medial prefrontal cortex, the nucleus

accumbens and the ventrolateral striatum using HPLC-ED. WKY rats demonstrated initial effort-related deficits in FR5/PR responding compared to SD controls, which ameliorated with training. Amphetamine significantly decreased FR5 work output, but increased responding on the PR phase in both SD and WKY rats. This effect was more pronounced in SD rats compared to WKY rats. In addition, sex differences were evident both in FR5/PR performance and in the behavioral response to amphetamine treatment. Moreover, females demonstrated higher levels of voluntary wheel-running than males. Finally, tissue concentrations of dopamine were lower in the NA and VLS of WKY compared to SD rats. Taken together, results suggest dysfunctions in mesolimbic DA neurotransmission in the WKY strain, likely underlying the depressive phenotype. The present study represents an important initial step in validating the WKY strain as a rat model of effort-related dysfunctions relevant to depression and other neuropsychiatric disorders.

Keywords: Depression, Dopamine, Effort, Motivation, Serotonin

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LIST OF ABBREVIATIONS

DA	Dopamine
DAT	Dopamine Transporter
DOPAC	3,4-Dihydroxyphenylacetic acid
ER	Estrogen Receptors
FR1	Fixed Ratio 1
FR5	Fixed Ratio 5
HPLC-ED	High-Performance Liquid Chromatograph with Electrochemical Detection
MAO	Monoamine Oxidase
mPFC	Medial Pre-Frontal Cortex
MSN	Medium Spiny Neurons
NA	Nucleus Accumbens
PR	Progressive Ratio
SD	Sprague-Dawley
VLS	Ventrolateral Striatum
VMAT	Vesicular Monoamine Transporter
VTA	Ventral Tegmental Area
WKY	Wistar-Kyoto
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	Serotonin

Chapter I

BACKGROUND AND SIGNIFICANCE

A. Introduction

Depression is a complex, multifaceted psychiatric disorder that affects millions of individuals worldwide. It is characterized by various affective, cognitive and motivational symptoms, including long-term feelings of unhappiness, anhedonia, deficits in attention, and cognitive inflexibility (Anxiety and Depression Association of America [ADAA], n.d.). Impairments in behavioral activation and effort-related functions of motivation, such as psychomotor retardation, fatigue, loss of energy, and reduced exertion of effort are critical and debilitating features of depression (Stahl 2002; Demyttenaere et al., 2005; Treadway and Zald 2011; Fava et al., 2014). Recent evidence emphasizes that most people with depression have fundamental deficits in behavioral activation and exertion of effort, and the severity of such motivational symptoms is correlated with problems of employment, social functioning, and treatment outcome (Tylee et al. 1999; Stahl 2002). Furthermore, motivational deficits are often resistant to first-line treatment options, such as selective-serotonin reuptake inhibitors (SSRI), and in some patients SSRIs can induce or exacerbate these symptoms (Fava 2014). Of note, effort-related motivational symptoms are present in multiple psychiatric and neurological disorders (Demyttenaere et al. 2005; Barch et al. 2014; Chong et al. 2015). Because of the clinical significance of these effort-related and activational symptoms of depression, along with the ever-increasing interest in identifying the neural circuitry related to individual psychiatric symptoms (i.e. NIH

Research Domain Criteria {RDoC}), it is critical to develop animal models that specifically study motivational processes.

Men and women differ in the occurrence and symptomatology of depression, as well as in their response to antidepressant treatment (Marcus et al. 2005; Wittchen et al. 2011). Women are twice as likely to be diagnosed with depression, and often report greater illness severity, and more symptoms than men. For instance, women are more likely to have hypersomnia and weight gain, and comorbid anxiety disorders (“Depression in women: Understanding the gender gap”, 2016; Smith et al., 2008). Depressed men are more likely to be successful than women when they attempt suicide, and show higher rates of anger, hostility, and substance abuse (Martin et al., 2013; Eaton et al., 2012). Despite these sex differences, most research on animal models of depression has been limited to the use of male rodents as experimental subjects. It is important that studies using animal models of depression include investigation of both male and female animals.

The neural basis of effort-related dysfunction in depression is complex, but there is a body of research implicating alterations in dopamine (DA) activity in discrete cortical and striatal structures (Caligiuri and Ellwanger et al. 2000; Schmidt et al. 2001; Tellez et al. 2008; Treadway and Zald, 2011). Specifically, nucleus accumbens DA has been implicated in the motivational aspects of motivation and effort-related processes, both in animals (Salamone and Correa 2012) and humans (Treadway et al. 2012a,b). Neuroimaging studies in depressed patients have identified alterations in DA neurotransmission, including changes in DA synthesis capacity as indexed by L-3,4-dihydroxyphenylalanine (L-DOPA) uptake, as well as changes in striatal distribution and availability of DA receptors, and DATs (Agren and Reibring 1994; Weintraub et al. 2005).

Tests of effort-related functions have been developed in humans (Treadway and Zald 2009), and recent studies demonstrated that individual differences in striatal DA transmission of healthy subjects were correlated with willingness to exert effort for larger rewards, in particular when reward probability was low (Treadway et al. 2012a). In contrast, people with major depression show reduced selection of high effort alternatives (Treadway et al., 2012b). Similarly, manipulations that impair DA transmission (i.e., by injection of DA antagonists or depleting agents) reliably reduce effortful behavior in rats, and alter effort-related decision-making, such that rats are biased towards the selection of low effort, less preferred alternatives (Farrar et al. 2010; Salamone and Correa 2012). It is important to note that these behavioral effects of interference with DA transmission do not produce effects similar to reinforcer devaluation by suppression of primary food motivation (Salamone and Correa 2002). In fact, rats will climb a barrier to obtain high-reward option when faced with either no reward alternative, or a second high-effort, but low-reward alternative (Yohn et al. 2015). Furthermore, alteration of DA transmission with antagonists, DA depletions, or genetic manipulations does not change hedonic reactivity to sucrose, preference for sucrose pellets (reinforcer for effort option) over chow in free-feeding choice tests, or consumption of sucrose pellets (Salamone and Correa 2012; Berridge and Kringelbach, 2015; Pardo et al., 2015). Rather, these DA manipulations alter the allocation of responses in a manner that interacts with ratio requirement of a given task, such as the amount of lever presses needed in order to obtain a food reinforcement (Salamone, 1986; Salamone et al., 2003). For instance, fixed ratio 1 (FR1) responding is minimally or not affected by manipulations that interfere with accumbens DA, while rats responding on moderate size ratio schedules (FR5, 16, 20) showed modest reductions in response rates,

and rats tested on schedules with high ratios (e.g., FR16, 64, 300 or progressive ratio: PR) were severely impaired (Caul and Brindle 2001; Salamone et al., 2001; Ishiwari et al., 2004). Taken together, these observations indicate that rats with compromised DA neurotransmission retain their cognitive and motor abilities needed to distinguish between stimuli, make decisions, and physically pursue their goals, but they have a low-effort bias to obtain food (Salamone and Correa 2012; Berridge and Kringelbach, 2015; Pardo et al., 2015).

Conversely, interventions that increase accumbens DA levels have been shown to cause the opposite effect, and increase behavioral activation (Delfs et al. 1990; De Jong et al. 2015). Stimulants that enhanced DA transmission, for example, will increase operant responding even on schedules that only generate low baseline rates of responding, an effect that is diminished with neurotoxic depletion of accumbens DA (Robbins et al. 1983; Delfs et al. 1990). Moreover, knockdown of ventral tegmental DA D2 autoreceptors, which enhance accumbens DA transmission, increased motivation for food as measured by PR responding (De Jong et al. 2015).

B. Objectives and Hypotheses

Recognizing the clinical significance of motivational symptoms of depression, and the substantial evidence indicating a critical role of monoamine neurotransmission in effort-related functions, the present study explored the use of the Wistar-Kyoto (WKY) rat model of depression to examine motivational dysfunctions. The WKY strain was initially developed as the normotensive control strain for the spontaneously hypertensive rat strain (Okamoto and Aoki 1963). Compared to many other strains, including the Sprague-Dawley

(SD), the WKY strain demonstrates a syndrome of hormonal, behavioral, and physiological dysfunctions that are relevant to human depression. For instance, WKY rats are hyper-reactive to stress and show dysregulation of the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–thyroid (HPT) axes (Solberg et al. 2001; Gómez et al. 1996). WKY male rats also show decreased activity in the conditioned defensive burying test and open field test, and increased immobility in the forced swim test compared with control Wistar or SD rats (Paré 1992, Paré 1994, Will et al. 2003), an effect that is reduced with administration of SSRIs (Detke 1997). Additionally, WKY rats display anhedonia-like phenotype as indicated by reduced preference to sucrose and other drug rewards (Rauhut et al. 2008). WKY also exhibit neurochemical abnormalities consistent with those observed in depressed patients, including altered serotonergic, noradrenergic and dopaminergic systems (Jiao et al. 2011). Also, previous work has demonstrated differential patterns of striatal dopamine transporters (DAT), along with D1, D2 and D3 DA receptors in WKY rats compared to control strains (Novick et. al., 2008.) These studies suggest dysfunctional DA transmission in WKY rats underlying their behavioral pathology. Despite being studied on several behavioral paradigms, WKY rats have yet to be assessed on tasks that specifically assess effort-related functions.

The first experiment investigated the impact of d-amphetamine on effort-related functions using a mixed FR5/PR schedule of reinforcement operant task. There are several variants of this task, but the specific procedure used in the present study involved an initial 10-minute FR5 component, with an unchanging moderate-effort response requirement, immediately followed by a 50-minute PR component. The PR schedule gradually increases the lever-pressing requirement to obtain consecutive food reinforcers and therefore

presents the animal with a gradually incrementing work-related cost. The highest ratio requirement completed during a single test session indicates the maximum effort a subject is willing to exert for a reinforcer, and is therefore considered to be a measure of motivation (Arnold and Roberts 1997; Bradshaw 2012, Hamill et al. 1999; Hodos 1961). One advantage of using this mixed FR5/PR procedure is that it allows for evaluation of performance on tasks with low and high-effort requirements in the same animals (Oakeshott et al. 2012). It was hypothesized that WKY rats would display impaired performance on the FR5/PR task, by completing lower ratio requirements than SD rats, indicative of deficits in motivation. Beyond testing for baseline motivational differences, the effect of increasing accumbens DA activity on performance in the mixed FR5/PR task was evaluated. This was accomplished by administration of d-amphetamine, which has been shown to affect DA release throughout the brain (Sulzer 1995, Kahlig et. al., 2005). Amphetamine causes DA release by reversing the action of DA transporters (DAT), and vesicular monoamine transporters (VMAT-2) (Kahlig et. al., 2005; Sulzer et al., 1995). Given this known effect of amphetamine on DAergic activity, it was hypothesized that this manipulation would ameliorate the motivational deficits of WKY rats tested on the mixed FR5/PR task. In terms of female vs. male performance, however, it was expected that female rats would display increased work output and heightened sensitivity to psychostimulants such as d-amphetamine. This result would be consistent with evidence demonstrating increased DAergic activity in female compared to male rats (Simpson 2012).

The second experiment examined voluntary wheel running in SD and WKY male and female rats. Given previous studies suggesting that the WKY model is less active

overall (Paré and Redei 1993), it was expected that the WKY rats would display decreased wheel running activity compared to SD rats. Consistent with previous studies, it was also expected that females would have higher activity levels than males (van Hest et. al., 1987).

With literature consistently implicating mesocorticolimbic monoamines in effort-related processes (Salamone and Correa 2012), including performance on the PR task (Randall et al., 2012, 2014), the third and final experiment assayed tissue levels of neurochemicals from striatal and cortical structures using HPLC-ED from SD and WKY male and female rats. Specifically, DA, DOPAC, DOPAC/DA, 5-HT, 5-HIAA, and 5HIAA/5HT were evaluated within the NA, ventrolateral striatum (VLS), and medial prefrontal cortex (mPFC).

This three-part study was designed to evaluate whether WKY rats demonstrate any form of effort-related motivational impairment. Through a series of behavioral tasks, the present study revealed differences in baseline behavioral activation aspects of motivation between the strains and sexes. By introducing the psychostimulant d-amphetamine to the mixed FR5/PR task, underlying neurobiological differences were highlighted. Finally, the neurochemical analysis by HPLC-ED further explored these differences to help define the WKY phenotype more completely.

Chapter II

EFFORT-RELATED MOTIVATIONAL DYSFUNCTIONS: BEHAVIORAL AND NEUROCHEMICAL STUDIES OF THE WISTAR-KYOTO RAT MODEL OF DEPRESSION

A. Introduction

Depression is a complex, debilitating psychiatric disorder that affects more than 16.1 million adults of the U.S. population age 18 and older in a given year (ADAA Stats). Clinicians have come to emphasize the importance of motivational symptoms of depression related to behavioral activation and effort-related functions, such as psychomotor retardation and fatigue (Tylee et al., 1999; Stahl 2002; Demyttenaere et al. 2005; Treadway and Zald 2011; Fava 2014). Recent evidence emphasizes that most people with depression have fundamental deficits in behavioral activation and exertion of effort (Treadway and Zald 2011). Furthermore, motivational deficits are often resistant to first-line treatment options, such as selective-serotonin reuptake inhibitors (SSRIs) (Stahl 2002; Demyttenaere et al. 2005; Treadway and Zald 2011; Fava et al. 2014 and Fava 2014). As a result, people being treated for depression often continue to experience difficulty maintaining employment and functioning normally in society, thereby worsening their underlying depression (Tylee et al. 1999; Stahl 2002). Because of the clinical significance of these effort-related symptoms, and the growing emphasis on identifying neural circuits related to specific psychiatric symptoms (i.e., NIH Research Domain Criteria [RDoC], n.d.), it is critical to develop animal models that specifically assess motivational processes.

Brain dopamine (DA), particularly in the nucleus accumbens (NA), has been implicated in activational aspects and effort-related aspects of motivation (Caligiuri and Ellwanger et al. 2000; Schmidt et al. 2001; Tellez et al. 2008; Treadway and Zald 2011; Salamone and Correa 2012). Rats with accumbens DA depletions are very sensitive to the size of the ratio requirement in operant schedules of reinforcement (Aberman and Salamone 1999; Salamone et al., 2003). Moreover, interference with accumbens DA transmission (i.e., by injection of DA antagonists or depleting agents) reduces effortful behavior in rats, and alters response allocation in tasks that assess effort-related choice behavior (Farrar et al. 2010; Salamone and Correa 2012). In contrast, interventions that increase accumbens DA levels have been shown to cause the opposite effect and increase behavioral activation, leading to substantial and persistent work output in instrumental actions (Robbins et al., 1983; Delfs et al. 1990; De Jong et al. 2015).

Given the clinical significance of motivational symptoms of depression, and the growing evidence that monoamine neurotransmission is critical for effort-related functions, the present study explored the use of the Wistar-Kyoto (WKY) rat model of depression to examine motivational functions. The WKY strain was initially developed as the normotensive control strain for the spontaneously hypertensive rat strain (Okamoto and Aoki 1963). Compared to many other strains, including Sprague-Dawley (SD), WKY demonstrates a syndrome of hormonal, behavioral, and physiological dysfunctions that recapitulate aspects of human depression (Will et al., 2003; De La Garza and Mahoney, 2004; Solberg et al., 2001). Early work utilized traditional behavioral models of depression, including the forced swim and tail suspension tests. WKY male rats predominantly showed floating behavior and reduced struggle time compared to SD controls. Furthermore,

treatment with common antidepressants improved their responses, bringing WKY rats up to control levels (Detke et al., 1997; Paré 1994). WKY also exhibit neurochemical abnormalities consistent with those observed in depressed patients, including altered serotonergic, noradrenergic and dopaminergic systems (Jiao et al. 2011). Despite being studied on several behavioral paradigms, WKY rats have yet to be assessed on tasks that specifically assess effort-related functions.

The experiments in this study assessed whether the WKY rats demonstrate any form of effort-related motivational impairment. The first experiment examined differences in fixed ratio 5/progressive ratio (FR5/PR) responding and evaluated the behavioral effect of d-amphetamine on SD and WKY rats responding on a mixed FR5/PR schedule of reinforcement operant task. This mixed FR5/PR task involves an initial 10-minute FR5 component (fixed moderate-effort response requirement), immediately followed by a 50-minute PR session (escalating effort response requirement). Amphetamine has been shown to increase accumbens DA release by reversing the action of the DA transporters (DAT) and vesicular monoamine transporters (VMAT-2) (Sulzer et al., 1995). It was hypothesized that WKY rats responding on a mixed FR5/PR task would demonstrate motivational impairments that would be ameliorated by amphetamine treatment. To further characterize WKY's motivational functions, a second study examined voluntary wheel running in SD and WKY male and female rats. Because of the literature implicating mesocorticolimbic monoamines in effort-related processes (Salamone and Correa 2012), including performance on the PR task (Randall et al. 2012, 2014), this study also assayed tissue levels of DA, serotonin (5-HT), and their metabolites in relevant cortical and striatal structures of

SD and WKY male and female rats, using high-performance liquid chromatography with electrochemical detection (HPLC-ED).

B. Materials and Methods

1. Animals

Sprague-Dawley (SD) and Wistar-Kyoto (WKY) male and female rats were purchased from Charles River Laboratories (Kingston, NY, USA). Rats were housed in same-sex, same genotype pairs in cages and allowed at least one week of acclimation to the colony prior to any experimental manipulations. The colony was maintained at 23 °C, with a 12-hour light/dark cycle (lights on at 07:00 h). Rats (n=32) evaluated in the mixed FR5/PR task were food-restricted to 85% of their projected free-feeding body weights for operant training and testing, with water available ad libitum in their home cage. Rats were fed supplemental chow to allow weight gain throughout the experiment (see Appendix 1 for further information). All other rats used (wheel running, n=32, and neurochemical analysis n=32) had ad libitum access to rat chow and water. The methods and procedures of the present study were approved by the University of Massachusetts Amherst Animal Care and Use Committee and followed NIH guidelines.

2. Pharmacological Agent and Dose Selection

(+)- α -Methylphenethylamine (d-amphetamine) sulfate salt from Sigma–Aldrich (St. Louis, MO) was dissolved in 0.9% saline solution and injected intraperitoneally (IP) in a volume of 1 ml/kg body weight. On drug test days, rats received IP injections of either saline vehicle, or 0.5 or 1.0 mg/kg amphetamine, 20 min before FR5/PR testing. Drug

testing was conducted using a within-subjects design, with each rat receiving all treatments, in a pseudo-random, modified Latin square protocol. Each rat received each treatment condition twice, such that all rats received each treatment once before any treatment was repeated. Drug testing occurred on Tuesdays and Fridays, while baseline FR5/PR (i.e., non-drug) sessions continued on the other three days of each week: Mondays, Wednesdays and Thursdays. Doses and injection time were selected based on pilot studies and previous research (Mayorga et al. 2000). At this dose range, amphetamine effectively increases both extracellular DA levels in the accumbens and locomotor activity, an effect that is still detected at 75-90 minutes post-injection (Mayorga et al. 2000; Vandershuren et al. 1999; Zocchi et al. 1998).

3. Mixed FR5/PR Task

Operant sessions were conducted in eight sound-attenuated operant conditioning chambers (Med Associates, St Albans, VT, USA) with sessions 5d/week. Each chamber (30.5 x 24 x 21 cm³) had two retractable levers, located on either side of a central food receptacle into which high carbohydrate reinforcement pellets (Dustless precision pellets, purified, 45 mg, BioServ, Frenchtown, NJ, USA) were delivered by a pellet dispenser outside the chamber. Each chamber had one 100 mA house light on the wall opposite the food receptacle that was continuously lit during sessions. For all sessions, both levers were extended into the chamber, but only one lever was active (i.e., only responses to the active lever resulted in the delivery of a food reinforcer). The opposite lever was inactive and had no programmed consequence, although responses to the inactive lever were recorded.

Active lever assignment (left or right lever) was counterbalanced across experimental groups.

All behavioral testing occurred between 09:00 - 13:00 h. Rats were initially trained to lever press on a continuous Fixed Ratio 1 (FR1) reinforcement schedule for one week, whereby each press of the active lever was reinforced with one pellet. Training sessions lasted 30 minutes. The animals were then shifted to a FR5 schedule of reinforcement (30-min sessions). During FR5 training, one reinforcer was delivered for every fifth response on the active lever. These sessions continued until 90% of animals reached 500+ active lever presses. This was achieved by the ninth FR5 session, after which rats were trained on a Progressive Ratio (PR) schedule of reinforcement. PR sessions lasted 60 min. In this PR schedule, the number of active lever presses required to obtain one reinforcer is progressively higher with each successive reinforcer through the following series based on the exponential progression described by Roberts and Richardson (1996): 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, 1347, 1646, 2012, 2459, 3004.

After 2 weeks of PR training, rats were introduced to a mixed FR5/PR task. In this procedure, each session started with a 10-min FR5 schedule component, followed by a 50-min PR component (same PR schedule as previously). Rats were trained on the mixed FR5/PR task until they attained stable levels of lever pressing (18 sessions), after which drug testing began. On baseline and drug treatment days, rats typically consumed all of the operant pellets that were delivered from lever pressing during each session.

4. Voluntary Wheel-Running

SD and WKY males and females (n=8 per group) were individually housed and given access to running wheels (35.6 cm diameter, Med Associates, St. Albans VT) for 72 hrs. Activity data were captured using MEDPCIV, and included total number of revolutions, and timestamps for each revolution. Daily and cumulative total distances run were determined in kilometers by multiplying recorded wheel revolution counts by the circumference of the wheel.

5. Tissue Collection and High Performance Liquid Chromatography

A separate cohort of WKY and SD male and female rats (n=32) were anesthetized, and their brains rapidly extracted and immediately frozen. Bilateral tissue punches (1 mm diameter) were dissected from 1 mm thick cross sections from medial prefrontal cortex (mPFC), nucleus accumbens (NA), and ventrolateral striatum (VLS). Tissue samples were placed in pre-weighed 1.5 ml tubes, and stored at -80°C until further neurochemical analysis.

Tissue samples were homogenized in 0.1N perchloric acid + 1 mM EDTA-2Na at 20 $\mu\text{l}/\text{mg}$, and centrifuged at 16,000 rcf for 15 min at 4°C in filtered tubes. The resultant supernatants were assayed for monoamines and their metabolites, including dopamine (DA) and its metabolite, 3–4-dihydroxyphenylalanine (DOPAC) and serotonin (5-HT) and its metabolite 5-hydroxyindole acetic acid (5-HIAA), using high-performance liquid chromatography with electrochemical detection (HTEC-500, Eicom, San Diego, CA, USA). A Eicompak SC-30DS separation column (Eicom, San Diego, CA, USA) was used and the mobile phase consisted of 0.1 M citrate-acetate buffer, pH 3.5, 0.1 mM EDTA, 1.2

mM sodium octyl sulfate, and 20% (v/v) methanol. The electrochemical detector included a graphite electrode set at a voltage of 750 mV vs a Ag/AgCl reference electrode. The flow rate was set to 400 μ l/min.

C. Statistical Analysis

Data were analyzed with linear mixed models (SPSS v25), using the best-fitting covariance structure. For the behavioral pharmacology experiment, number of active and inactive lever presses, reinforcers earned, magazine entries, magazine entries per reinforcer, and highest ratio completed were analyzed. Between-subjects factors included genotype and sex, while drug treatment was included as the within-subjects factor. Data from each treatment condition were averaged for each rat before statistical analysis was performed. For the wheel running experiment, daily distance ran was analyzed. Within-subjects factors included light cycle (light, dark) and day. For the tissue neurochemical data, because male and female tissue samples were handled and processed differently, separate analyses of each sex were used to determine differences in DA and 5-HT levels, their metabolites DOPAC and 5-HIAA, and their DOPAC/DA, and 5HIAA/5HT ratios within the mPFC, NA and VLS. Follow-up tests of significant main and interaction effects were conducted as applicable. In all figures, data are expressed as mean \pm standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

D. Results

1. Mixed FR5/PR Training

Data from mixed FR5/PR training phase are presented in Figure 1, split into FR5 training and PR training. In the FR5 phase, there was a significant strain x session interaction ($F_{(1,27)}=5.190$, $p=0.031$, with WKY rats initially earning significantly fewer reinforcers than SD controls (Figure 1A). WKY rats increased the number of reinforcer earned with training, and by the end of training both strains earned similar number of reinforcers ($p=ns$). In addition, there was a marginally significant main effect of sex ($F_{(1,27)}=3.763$, $p=0.063$), with males earning more reinforcers than females throughout FR5 training (Figure 1A). In the PR training phase (Figure 1B), both strains earned similar number of reinforcers, but the number of reinforcers earned was reduced as training progressed (Session main effect: $F_{(1,27)}=8.591$, $p=0.07$).

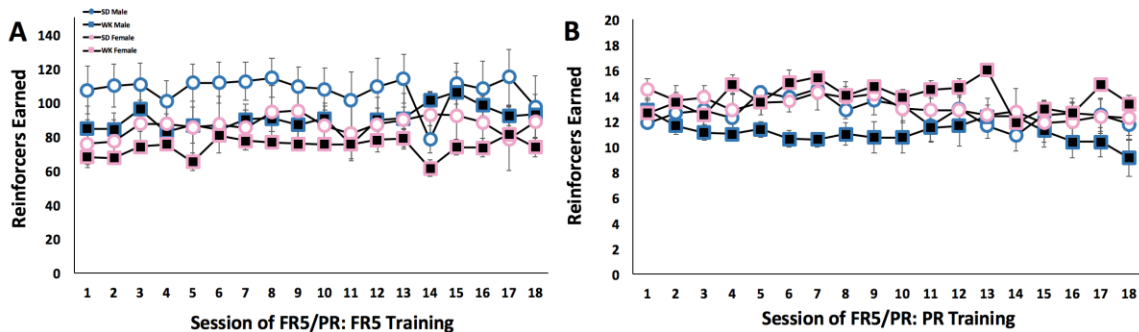


Figure 1. Mean (\pm SEM) number of reinforcers earned during mixed FR5/PR training across sessions in SD and WKY males and females. (A) Data from the 10 min FR5 training, (B) Data from the 50 min PR training.

D-amphetamine differentially decreased FR5 performance in SD and WKY rats

The effect of d-amphetamine (0, 0.5 and 1.0 mg/kg) on the FR5 phase of the mixed FR5/PR task is shown in Figure 2A-E. There were significant performance strain x treatment interactions for active lever presses ($F_{(2,52.19)}=4.675$, $p=0.014$), inactive lever presses

($F_{(2,32.84)}=3.519$, $p=0.041$) and reinforcers earned ($F_{(2, 52.188)}=4.69$, $p=0.013$). Amphetamine significantly decreased in a dose-dependent manner the number of active lever presses ($F_{(2,52.19)}=47.11$, $p<0.001$; Figure 2A), the number of reinforcers earned ($F_{(2,52.19)}=47.20$, $p<0.001$; Figure 2B), and the number of magazine entries ($F_{(2,37.27)}=12.51$, $p<0.001$; Figure 9D). Post-hoc comparisons revealed that the total active lever presses, reinforcers earned and magazine entries were all decreased in SD rats at all doses compare with vehicle ($p<0.05$), whereas these measures were only decreased in WKY rats at 1.0 mg/kg amphetamine. In addition, amphetamine significantly increased the number of inactive lever presses ($F_{(2,32.84)}=4.39$, $p=0.02$; see Figure 2C), with SD females displaying higher inactive lever presses than all other groups ($p<0.05$).

In addition, there were sex differences in the number of active lever presses ($F_{(1, 26.80)}=9.32$, $p<0.006$; Figure 2A), number of reinforcers earned ($F_{(1, 26.80)}=9.34$, $p<0.006$; Figure 2B), and number of magazine entries per reinforcer ($F_{(1,33.76)}=5.26$, $p<0.03$; Figure 2E), with males exhibiting overall higher levels of responding than females ($p<0.05$; Figure 2A-E).

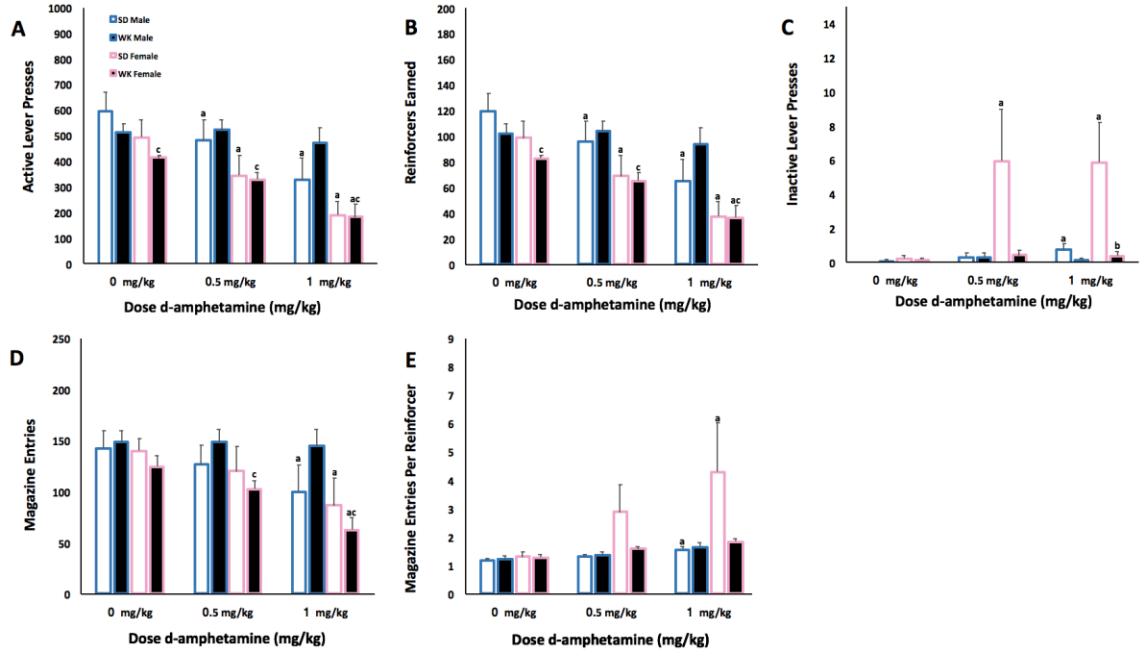


Figure 2. Effect of d-amphetamine on FR5 performance in SD and WKY males and females during mixed FR5/PR testing. Mean (\pm SEM) values for (A) active lever presses, (B) reinforcers earned, (C) inactive lever presses, (D) magazine entries, and (E) magazine entries per reinforcer. ^a $p < 0.05$ within-subjects, relative to vehicle, ^b $p < 0.05$ between strains, relative to control SD, ^c $p < 0.05$ within strain, between male and female groups.

D-amphetamine differentially increased PR performance in SD and WKY rats. The effect of amphetamine on the PR performance of the mixed FR5/PR is shown in Figure 3A-E. Amphetamine significantly increased the number of active lever presses ($F_{(2,38.51)}=16.79, p < 0.001$; Figure 3A), with both SD and WKY rats displaying increased active lever pressing rates following either dose of amphetamine relative to saline ($p < 0.05$). Similarly, 0.5 and 1.0 mg/kg amphetamine increased the number of reinforcers earned ($F_{(2,56)}=18.59, p < 0.001$; Figure 3B), the number of magazine entries ($F_{(2,32.25)}=7.75, p < 0.003$; Figure 3E) and the highest ratio completed ($F_{(2,56)}=16.29, p < 0.001$; Figure 3C) in both SD and WKY rats relative to saline ($p < 0.05$). A significant performance strain x drug treatment interaction for inactive lever presses ($F_{(2,33.68)}=4.794, p = 0.015$) and magazine entries per reinforcer ($F_{(2,33.59)}=3.594, p = 0.038$), indicated that only SD rats'

scores increased significantly with doses of amphetamine compared with WKY rats ($p<0.05$)(Figure 3F).

There was a significant main effect of sex for inactive lever presses ($F_{(1,34.38)}=6.764$, $p<0.014$), magazines entries ($F_{(1,21.00)}=5.32$, $p=0.031$; Figure 3F), and magazine entries per reinforcer ($F_{(1,23.27)}=10.51$, $p=0.004$, with females exhibiting higher scores than males ($p<0.05$). A significant performance sex x treatment interaction for active lever presses ($F_{(2,38.51)}=5.99$, $p=0.005$), inactive lever presses ($F_{(2,33.68)}=3.09$, $p=0.058$), reinforcers earned ($F_{(2,56)}=13.88$, $p<0.001$), and magazine entries ($F_{(2,32.23)}=3.11$, $p<0.058$), indicated that female rats had an inverted-U-shaped response following amphetamine treatment, whereas males had a dose-response increase in performance ($p<0.05$) (Figure 3A-F).

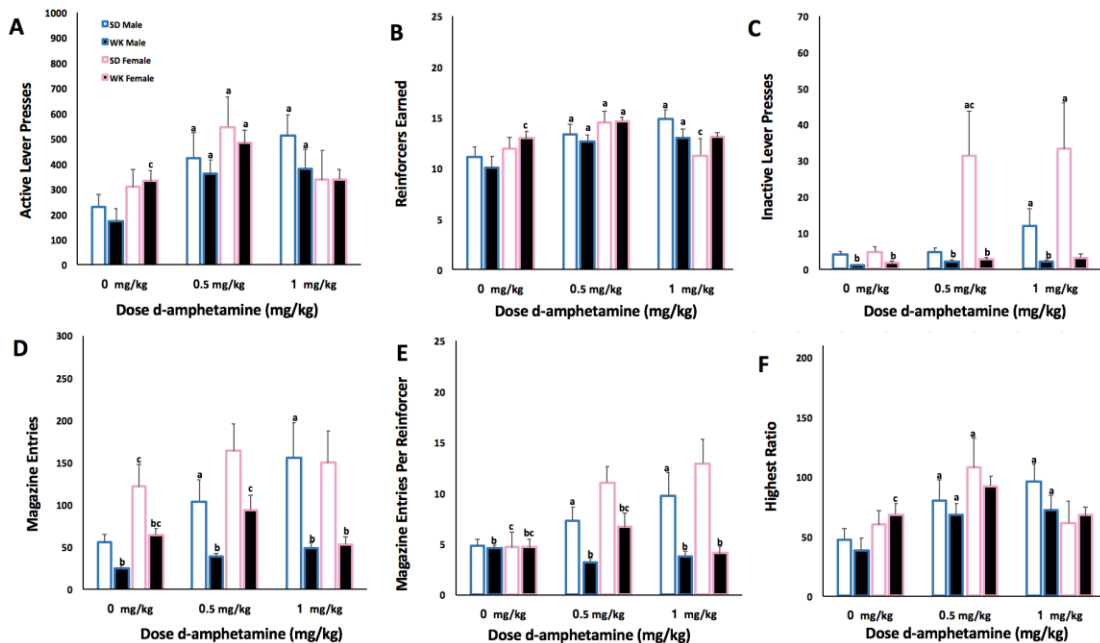


Figure 3. Effect of d-amphetamine on PR performance in SD and WKY male and female rats during mixed FR5/PR testing. Mean (\pm SEM) values for (A) active lever presses, (B) reinforcers earned, (C) inactive lever presses, (D) magazine entries, and (E) magazine entries per reinforcer, (F) highest ratio completed. ^a $p<0.05$ within-subjects, relative to vehicle, ^b $p<0.05$ between strain, relative to control SD, ^c $p<0.05$ within strain, between male and female groups.

2. Voluntary Wheel Running

To first gain an overview of voluntary wheel use, the cumulative total distances run over the 72-h period were calculated. There was a significant difference in running distance between males and females ($F_{(1, 28)}=4.357$, $p=0.046$), but not between strain ($F_{(1, 28)}=0.621$, $p=ns$), with female rats running almost twice as much as males over a 72 h-period (Figure 4A). To assess changes in running behavior over time, a detailed analysis of wheel running was conducted according to the light/dark phases of the light cycle. There was a marginally significant effect of sex ($F_{(1, 28)}=4.089$, $p=0.053$), dark/light cycle ($F_{(1, 28)}=25.305$, $p<0.001$), as well as a marginally significant sex x dark/light cycle interaction effect ($F_{(1, 28)}=3.656$, $p=0.066$). No strain ($F_{(1, 28)}=0.243$, $p=ns$) effects were observed. All groups ran longer distances during the dark phase of the light/dark cycle, with females running significantly more than males (all $ps<0.05$). In addition, an increase in daily distance ran occurred from the first day of wheel exposure in both SD and WKY rats ($F_{(1,28)}=19.525$, $p=0.001$; Figure 4B), with increased running distance on night 3 compared to night 1 (all $ps<0.05$).

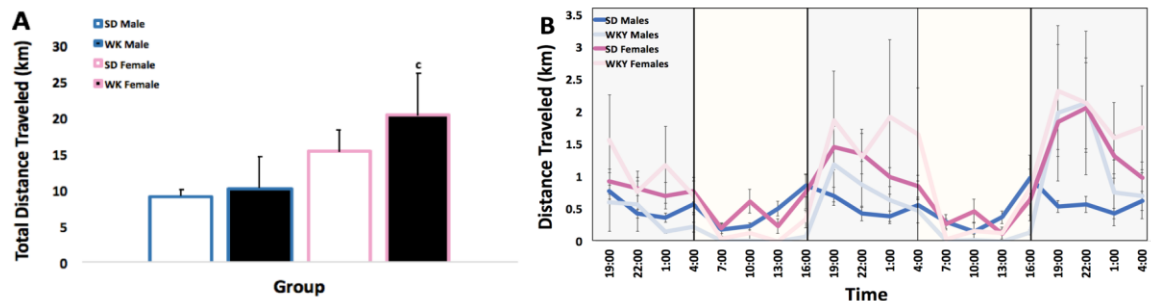


Figure 4. Voluntary wheel running in SD and WKY male and female rats. (A) Cumulative total distance (km) ran in SD and WKY male and female rats in 72 h of running wheel access (B) Distribution of distance ran in 3 h time bins over 72h according to the light/dark cycle. Mean \pm SEM. ^c $p<0.05$ within strain, between male and female groups.

3. Neurochemical Analysis

Tissue concentrations of DA and 5-HT and their metabolites (pg/mg tissue) in NA, VLS and mPFC of female and male rats are shown in Figures 5 and 6, respectively.

WKY female rats had lower dopamine levels in the NA and VLS compared to SD females. Differences in DA levels between strains were found in the NA ($F_{(1,12)}=5.63, p=0.035$) and the VLS ($F_{(1,12)}=6.66, p=0.024$), with higher DA levels in SD compared to WKY females (Figure 5). Concentrations of 5-HIAA also differed between strains in the NA ($F_{(1,12)}=7.24, p=0.02$) and the VLS ($F_{(1,12)}=5.60, p=0.036$), with higher 5-HIAA levels in SD than WKY females. In addition, 5-HT concentrations in the VLS were marginally different between SD and WKY females ($F_{(1,12)}=4.087, p=0.066$). Lastly, only in the VLS, significant differences in DOPAC/DA ratio were found between strains ($F_{(1,12)}=8.86, p<0.02$; Figure 5), with higher DA turnover in WKY females compared to SD females.

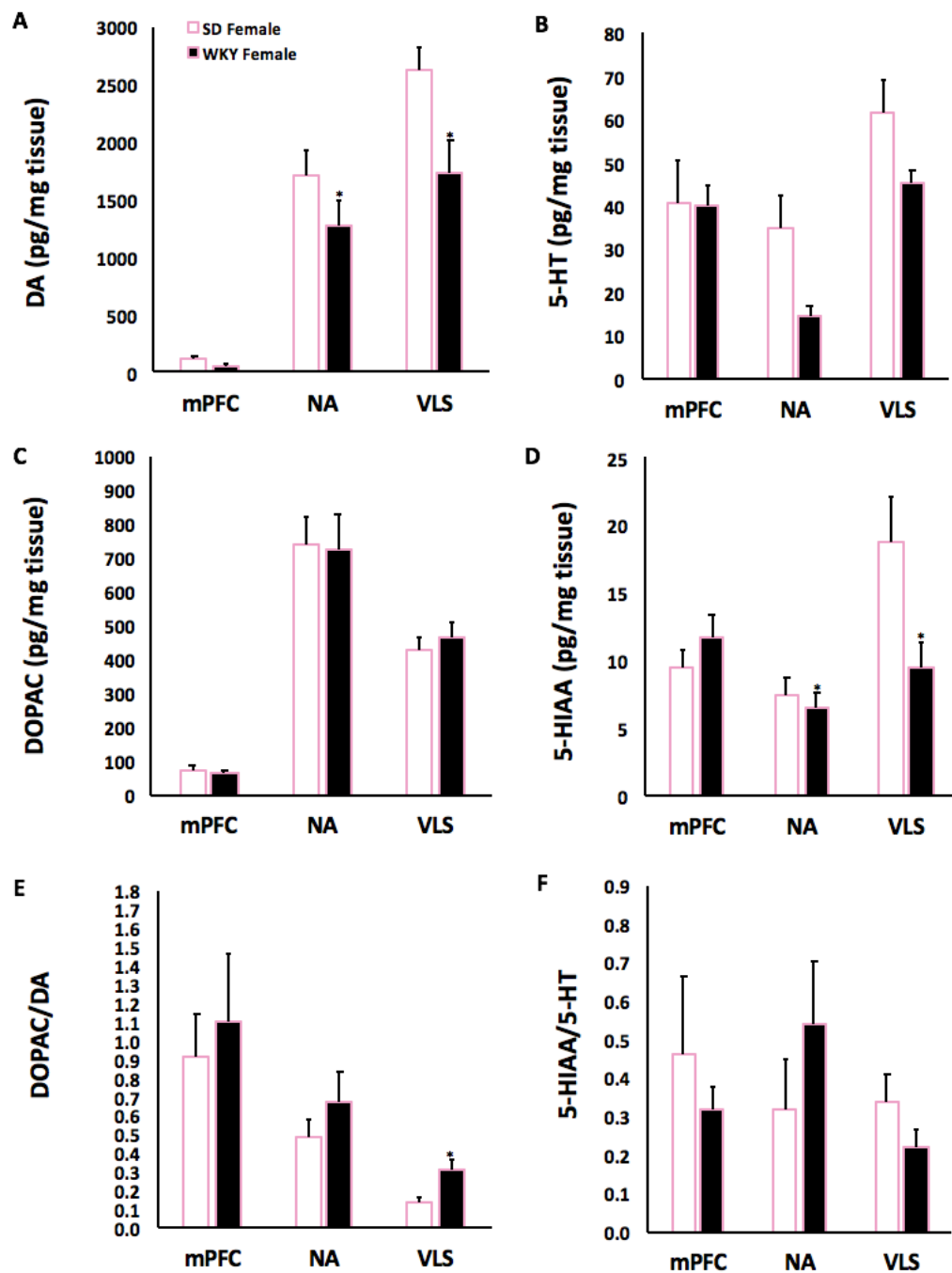


Figure 5. Tissue concentrations (pg/mg tissue) of (A) DA and (B) 5-HT, and their respective metabolites (C) DOPAC and (D) 5-HIAA, as well as their (E) DOPAC/DA ratio and (F) 5-HIAA/5-HT ratio within the mPFC, NA, and VLS of SD and WKY female rats. * $p < 0.05$ relative to SD females.

WKY male rats had lower accumbens monoamine levels compared to SD males

As shown in Figure 6, multivariate analysis revealed marginally significant differences in NA DA ($F_{(1,13)}=4.229$, $p<0.06$) and NA 5-HT ($F_{(1, 13)}=3.903$, $p<0.07$) between WKY and SD males. No strain differences were found for the neurotransmitter/metabolite ratios between WKY and SD males.

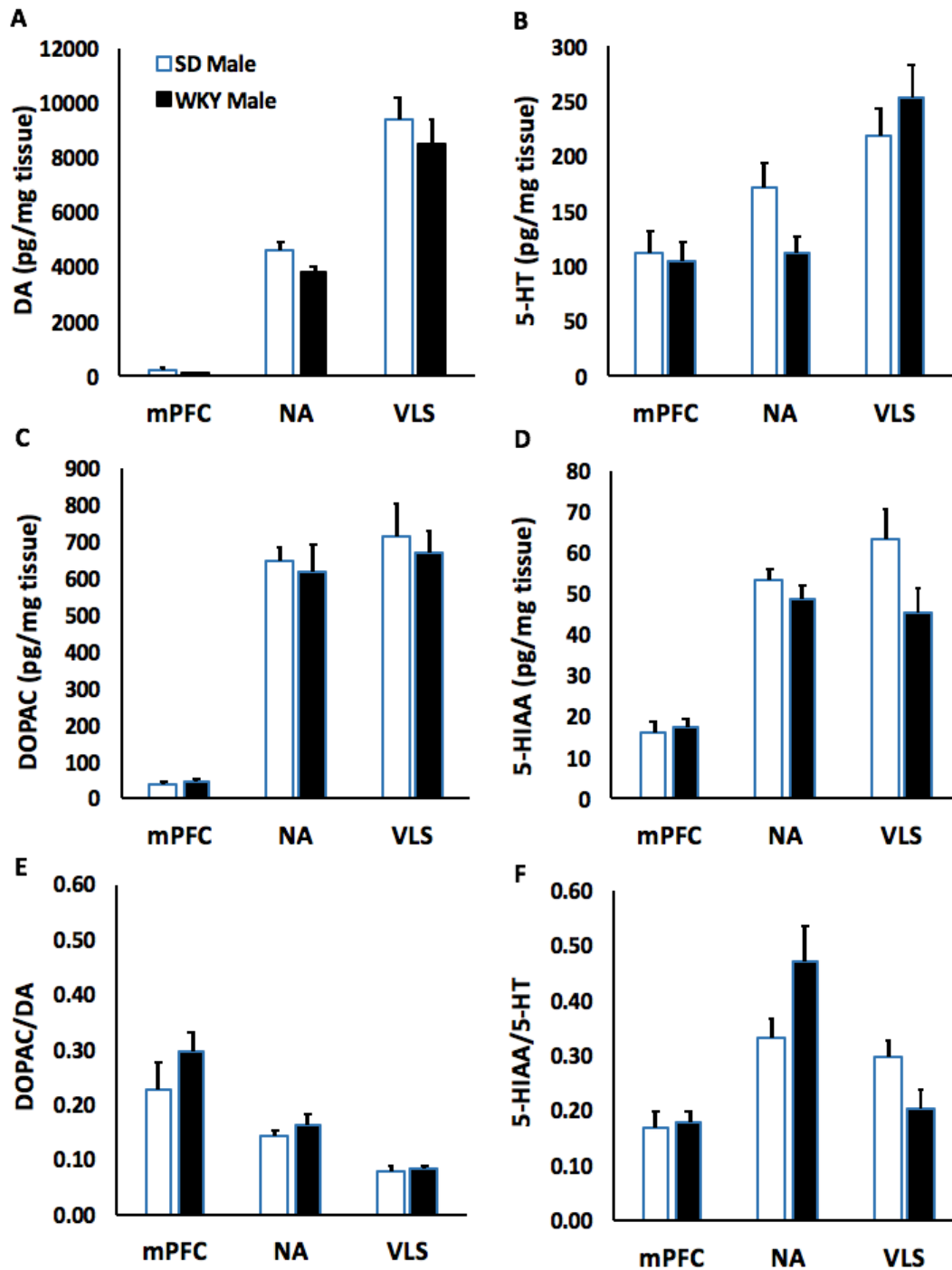


Figure 6. Tissue concentrations (pg/mg tissue) of (A) DA and (B) 5-HT, and their respective metabolites (C) DOPAC and (D) 5-HIAA, as well as their (E) DOPAC/DA ratio and (F) 5-HIAA/5-HT ratio within the mPFC, NA, and VLS of SD and WKY male rats.

E. Discussion

The findings of the present study demonstrated that the WKY rat strain exhibits behavioral and neurochemical alterations that are consistent with effort-related dysfunctions. The psychostimulant amphetamine impacted performance in the FR5/PR task in a strain-dependent manner, with WKY rats displaying decreased responses to the behavioral effects of amphetamine. The current results also showed significant sex differences in response to amphetamine, with females exhibiting a greater behavioral response than males. Moreover, females demonstrated higher levels of voluntary wheel-running than males. Finally, tissue concentrations of dopamine were lower in the NA and VLS of WKY compared to SD rats.

WKY rats demonstrated initial effort-related deficits in FR5/PR responding compared to SD control. With training, work output performance in SD decreased, reducing difference in performance between strains. One possible explanation is that the present study incorporated an overly-challenging PR schedule of reinforcement that proved to be too difficult for all groups. In this sense, a previous study in the lab using an easier PR schedule of reinforcement demonstrated significant differences between SD and WKY male rats that persisted over time (Farrar et al. 2016). Additional factors that may have contributed to the lack of difference between strains are pair-housing and food restriction procedures used in the present study, each of which has been shown to ameliorate depressive-like symptoms in rodents (LeTendre 2009; Lutter et al. 2008; Zhang et al., 2015).

Amphetamine impacted work output in a task-dependent manner. During moderate-effort FR5 testing, amphetamine decreased active lever presses and reinforcers earned, whereas during high-effort PR testing, d-amphetamine increased active lever presses, reinforcers earned, and highest ratios completed. These findings are consistent with previous studies showing that the impact of DA manipulations on instrumental behavior depends upon the task requirement or reinforcement schedule (Salamone et al. 2016). FR5 schedule of reinforcement produces a high rate of responding along with a short post-reinforcement pause. One possible reason for this asymmetry of amphetamine effect is that rats responding on FR5 were responding at or near their maximal response levels, meaning that subjects could only either show the same level of performance, or a reduction in performance with amphetamine. Another possible reason is that amphetamine reduced lever pressing in part because the motor stimulant effects of amphetamine is incompatible with this behavior. Also, it is well documented that amphetamine suppresses appetite under a broad range of conditions (Leibowitz and Rossakis 1978). However, the fact that amphetamine increased PR performance argues against this possibility. Unlike the FR5 schedule, rats' response rates on PR schedules decreases at large ratio values, and post-reinforcement pause length increases with requirement. Under this schedule, amphetamine has been shown to increase response rate by decreasing long post-reinforcement pauses (Bailey et al., 2015; Mayorga et al. 2000).

Amphetamine also impacted work output in a strain-dependent manner. Specifically, amphetamine was more effective in altering FR5/PR performance in SD rats than WKY rats. Furthermore, there was a selective increase in the number of inactive lever presses and magazine entries per reinforcer in SD rats following amphetamine treatment,

indicative of increased motor activity. This result is consistent with previous findings demonstrating increased motor activity with acute amphetamine treatment, due to increased extracellular DA release following amphetamine blockade of DATs and VMATs (McNamara et al. 1993; Minassian et al., 2016). This heightened sensitivity to the behavioral effects of amphetamine on SD versus WKY rats suggests alterations in monoamine signaling, especially DA neurotransmission, underlying effort-related functions, including reduced number of DATs, VMATs and/or reduced number of postsynaptic DA receptors. In this sense, previous studies demonstrated differences in striatal DATs, DA D1 and D2 receptor binding in WKY rats compared to control strains (Jiao et al. 2003; Yaroslavsky et al. 2006; Novick et al. 2008). When these present results are taken in the context of previous findings, the reduced behavioral effects of amphetamine in WKY rats might further suggest deficits in accumbens DA activity.

This study also revealed differences in the behavioral response to amphetamine in male and female rats. In contrast to males, 1.0 mg/kg amphetamine disrupted work output in FR5/PR task in females. Sex differences in the behavioral response to amphetamine were also reflected in the increased number of inactive lever presses by SD females. In agreement with previous studies that have found that female rats are generally more sensitive to the effects of d-amphetamine than males (Eubig 2014; Becker and Hu 2008; Weiss et al. 2015), our results suggest neurobiological differences between males and females. Increased metabolism of amphetamine in male rats could contribute to the attenuated behavioral response observed in male rats. However, previous studies demonstrated that these behavioral sex differences in response to amphetamine persist even with equivalent brain concentrations of amphetamine (Becker et al. 1982). Intrinsic

variations in monoamine function could partially account for the increased responsiveness to amphetamine seen in females. In this sense, amphetamine-induced DA release in the striatum has been found to be greater in females than males (Castner et al. 1993; Becker 1999), indicative of increased DAT and VMAT2 function (Morissette and Di Paolo 1993; Bhatt and Dluzen 2005). In addition, sex differences in the number of DA and 5-HT receptors have been identified (Zhand et al. 1999). Furthermore, previous studies have revealed that female rats possess greater dendritic spine density on NA medium spiny neurons (MSN) (Forlano et al. 2010). These functional and structural differences likely contribute to greater baseline effort-related performance and heightened sensitivity/response to psychostimulants such as d-amphetamine observed in females. Circulating ovarian hormones, especially estradiol, in female rats have been shown to modulate the activity of monoamine systems, such as by influencing the excitability of DA neurons in VTA, DA synthesis and release (Zhang et al. 2008, Satta et al. 2018). Additionally, estrogen receptors (ERs) are highly expressed in NA and many of its afferents, which may contribute to estradiol's effects on psychomotor behaviors, and/or affect NA MSN structure and function (Almey et al. 2015; Satta et al. 2018).

Voluntary wheel running took place mostly during the active phase (dark phase of the light/dark cycle) in both SD and WKY rats. WKY strain was generally more active, although this difference didn't reach statistical significance. Although this finding seems contradictory, the fact that WKY rats run similar distances as SD rats, which have been found to be hypoactive in the running wheels (Ferguson and Cada 2003) further characterizes their depressive phenotype of WKY strain. In support of this, WKY rats are hypoactive in the open field task (Paré 1994).

Voluntary wheel running activity was higher in females than males, consistent with previous studies (Eikelboom and Mills 1988; Jones et al. 1990). Motivation to run has been linked to DA neurotransmission, and blockade of accumbens DA D2 receptors reduces running activity (Ebada, Kendall and Pardon 2016), suggesting sex-differences in DA function mediating this effort-related behavior. It has also been suggested that females are more efficient runners (in terms of energy expenditure) than males (Rezende et al. 2009; Rosenfeld 2017). Furthermore, differences in respiratory capacities, food intake and/or energy metabolism could mediate sex-related differences in the activity level (Schulz et al. 2002; Palmer et al. 2013; Novak et al. 2012).

Consistent with previous findings (De la Garza and Mahoney 2004; Scholl et al. 2010), neurochemical analysis with HPLC-ED indicated strain differences in tissue concentrations of monoamines. For instance, lower tissue levels of DA and higher DOPAC:DA ratios, along with lower tissue levels of 5-HIAA and 5-HT with higher 5-HIAA:5-HT ratios, within relevant striatal structures were found in WKY compared to SD rats. These findings further support the notion of altered DA and 5-HT neurotransmission in WKY rats. Specifically, our results are consistent with increased MAO activity in WKY rats. In the rat brain, both subtypes A and B of MAO are expressed in similar proportions (Youdim and Finberg 1983), but MAO-A is the predominant form responsible for both DA and 5-HT metabolism, converting DA into 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-HT into 5-hydroxyindolacetic acid (5-HIAA).

The degree to which differences in intracellular concentrations of monoamine neurotransmitters and their metabolites contribute to the behavioral phenotype of WKY remains unclear. For instance, findings from studies of Parkinson's Disease pathogenesis

have indicated that striatal DA depletions must be substantial (DA levels <20% of control) in order to result in Parkinsonian symptoms (Hornykiewicz, 2001). In the present work, intracellular levels of DA in WKY female rats is approximately 57% (within the mPFC), 65% (within the VLS), and 75% (within the NA) of SD controls, making it unlikely that intracellular levels of DA alone account for the observed phenotypical differences between the strains. Ongoing work in the Pereira lab is utilizing *in vivo* microdialysis to investigate the possibility that both basal and amphetamine-evoked extracellular levels of DA and 5-HT may nevertheless be reduced in WKY relative to SD rats, potentially reflecting alterations in vesicular release machinery.

The present study represents an important initial step in validating the WKY strain as a rodent model of effort-related dysfunctions related to depression. Specifically, the present results support potential dysfunction of DA pathways likely mediating the depressive phenotype of the WKY strain. Reduced sensitivity to the behavioral effects of amphetamine and altered intracellular concentrations of DA and 5-HT concentration in striatal structures are especially revealing of the potential mechanisms underlying WKY's behavioral abnormalities. Future studies should evaluate the WKY strain in other tasks that examine effort-related functions, including effort-related choice behavior such as the concurrent fixed-ratio 5 (FR5)/chow feeding choice procedure (Farrar et al. 2007).

As future studies continue to explore the cause and treatment options of depression, it is important for researchers to remain aware of sex-differences in neuropsychiatric disorders. The tendency for females to have a heightened motivational state and sensitivity to dopaminergic manipulation, as indicated by the progressive ratio task and as a result of apparent biological differences, highlights the importance of their inclusion in scientific

studies. By including both male and female subjects in future studies of the WKY model, it will be possible to gain a more complete understanding of the neurobiological origin of motivational deficits. In this way, new treatment options will ultimately be able to be developed in an effort to combat these highly debilitating core symptoms of depression.

Appendix A

METHOD OF PROJECTING BODY WEIGHT

A. Introduction

While working with rodents, it is often necessary to food-restrict them in order to encourage their engagement in behavioral tests. Fully-satiated rats will be uninterested in learning to perform tasks, or maintain exert effort, in order to receive food reinforcers during operant experiments, for example. By food-restricting their subjects, researchers can easily avoid this obstacle.

Studies that use food restriction as motivators often maintain animals at 85% of their ad libitum or age-based free feeding body weight. To achieve a reduction to 85% baseline weight, rats receive 5g/day per 100g body weight (~80-85% normal intake), with an expectation that target weight (85% ad lib) is reached in approximately one week. Daily visual inspection during weighting (coat quality, eye/nose secretions, demeanor/posture, body score) are and these notes are included in weight records.

As part of the present study, a method was developed to correctly determining age-adjusted base body weight in rats being food-restricted. This was accomplished by creating a growth curve from weights taken daily from animals in the Pereira lab to predict how much a rat would weigh if it had been maintained with ad libitum access to food.

The projected free-feeding weight was determined for each individual, based on their expected growth determined from control cohorts of SD and WKY rats housed in the laboratory (see Figure A1). Body weights were recorded daily from control WKY and SD males and females, and averaged for each strain and sex combination. For each group,

mean body weights were plotted as a function of age, and the best-fitting polynomial functions of the resulting plots were derived (Figure A1). The resulting functions were utilized daily to determine the projected free-feeding weights of each experimental, food-restricted animal, accounting for its age and baseline weight prior to food restriction. Supplemental chow was provided daily following operant training sessions, and the amount was determined on the basis of food earned during the operant session, the weight of the animal, and whether the animal needed to gain or lose weight to be maintained at the target weight (85% of projected free-feeding weight). If one cagemate needed to gain a considerable amount of weight, while the other needed to remain the same or lose weight, the pair was separated and fed individually, until both body weight percentages normalized. This typically occurred within a 24-hour period.

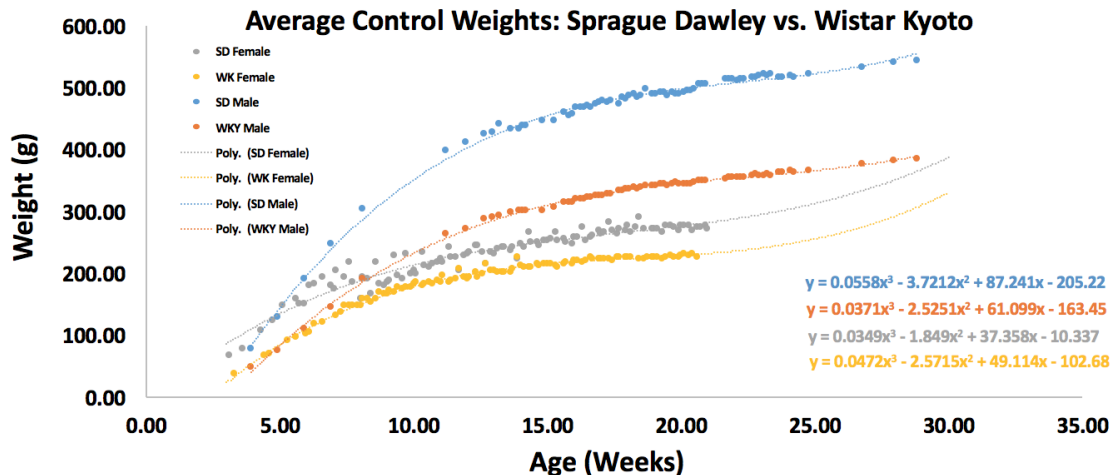


Figure A1. Mean \pm SEM body weight (grams) of male and female SD and WKY rats fed ad libitum.

B. Proof of Concept & Sample Calculation

Control SD and WKY male and female rats (n=4 per group) in the relevant age range and with recorded daily body weights over an extended period of time were

randomly chosen and use to demonstrate the accuracy of our projected weight and body weight percentage calculations.

Subject: WK57

Sex: Female

Strain: WKY

Equation: $y = 0.0472x^3 - 2.5715x^2 + 49.114x - 102.68$

Baseline Weight (g) = 141.92

Baseline Age (Weeks) = 7.57

Current Weight (g) = 160.78

Current Age (Weeks) = 8.71

$$\frac{\text{Expected Weight of WKY Female at Given Age}}{\text{Average WKY Female Weight at Baseline}} = \text{Projected Growth Factor}$$

$$\left(\frac{0.0472 * (8.71)^3 - 2.5715 * (8.71)^2 + 49.114 * (8.71) - 102.68}{0.0472 * (7.57)^3 - 2.5715 * (7.57)^2 + 49.114 * (7.57) - 102.68} \right)$$

$$\frac{161.21}{142.23} = 1.13 = \text{Projected Growth Factor}$$

$$\begin{aligned} &(\text{Projected Growth Factor}) * (\text{Subject Baseline Weight}) \\ &= \text{Subject's Projected Weight} \end{aligned}$$

$$(1.13) * (141.92) = \text{Subject's Projected Weight}$$

$$160.86 = \text{Subject's Projected Weight}$$

$$\left(\frac{\text{Subject's Actual Weight}}{\text{Subject's Projected Weight}} \right) \times 100 = \text{Body Weight Percentage}$$

$$\left(\frac{160.78}{160.86} \right) \times 100 = \text{Body Weight Percentage}$$

$$99.95 = \text{Body Weight Percentage}$$

As seen by this calculation, our process predicts that this non-food restricted, control, WK female subject would weigh 160.86 g at 8.71 weeks of age. This prediction is only 0.08g off of her actual weight at the given age.

Table 1: Proof of concept body weight projections.

Rat ID	Group	Age for Baseline Weight (Weeks)	Baseline Weight (g)	Age for Calculation (Weeks)	Growth Factor	Projected Weight at Given Age (g)	Actual Weight at Given Age (g)	Accuracy (%)
WK57	WKY Female	7.57	141.92	8.71	1.13	160.86	160.78	99.95
WK58	WKY Female	7.57	141.82	8.71	1.13	160.26	164.62	97.35
WK47G2	WKY Female	7.57	147.3	8.71	1.13	166.45	169.58	98.15
WK47G4	WKY Female	7.57	136.74	8.71	1.13	154.52	160.29	96.40
SD31E8	SD Female	16.3	310.74	21	1.09	338.99	324.75	95.80
SD55	SD Female	16.3	261.14	21	1.09	284.88	277.39	97.37
SD56	SD Female	16.3	248.97	21	1.09	271.61	264.73	97.47
SD23G4	SD Female	9.7	213	11.9	1.10	234.60	239.00	90.79
SD11A1	SD Male	12.6	444	22.2	1.22	543.77	529.14	97.31
SD11A2	SD Male	12.6	448.9	22.2	1.22	549.77	548.68	99.80
SD11A3	SD Male	12.6	441.1	22.2	1.22	540.22	521.07	96.45
SD11A4	SD Male	12.6	410.8	22.2	1.22	503.11	491.22	97.64
WK11A1	WK Male	13.9	297	15.6	1.06	315.41	312.44	99.06
WK11A2	WK Male	13.9	286.6	15.6	1.06	304.36	299.62	98.44
WK11A3	WK Male	13.9	285.5	15.6	1.06	303.20	299.00	98.62
WK11A4	WK Male	13.9	290	15.6	1.06	307.97	304.05	98.73

Appendix B

INITIAL TRAINING

A. Magazine Training

Both SD and WKY rats learned to use the appropriate lever during magazine training, although performance was not equal across groups. This consistent learning across sessions is indicated by the overall significant effect of Session on active lever presses ($F_{(5, 39.63)}=12.32, p<0.0001$). While male rats tended to have higher active lever presses than their female counterparts (main effect of Sex, $F_{(1, 39.08)}=4.9, p<0.05$), a significant Strain \times Sex \times Session interaction ($F_{(5, 39.63)}=3.52, p<0.05$; Figure B3A) indicated that male SD rats exhibited more active lever presses than male WKY rats on Session 3, while female SD rats exhibited more active lever presses than their WKY counterparts on Session 1 ($F_{(1, 23.4)}=5.95, p<0.03$; Figure B1A).

A gradual decline in inactive lever presses was observed across sessions as all rats began to learn which one of their two levers was active, as indicated by the significant main effect of Session on inactive lever presses ($F_{(5, 120.85)}=5.12, p<0.001$). A significant Strain \times Session interaction, however, revealed that this effect was only observed in SD rats ($F_{(5, 120.86)}=6.10, p<0.001$; Figure B1C), and not WKY rats ($F_{(5, 120.79)}=1.99, p=ns$).

As expected, given the pattern seen in active lever presses, there was a significant main effect of Session on reinforcers earned ($F_{(5, 39.77)}=12.24, p<0.001$), indicating that overall this measure increased across sessions. While male rats tended to earn more reinforcers than females (main effect of Sex, $F_{(1, 39.07)}=4.93, p<0.04$), a significant Strain \times Sex \times Session interaction ($F_{(1, 26.50)}=6.10, p<0.03$; Figure B3B) indicated that male SD

rats earned more reinforcers than male WKY rats on Session 3, while female SD rats earned more reinforcers than their WKY counterparts on Session 1 ($F_{(1,23.42)}=5.99$, $p<0.03$; Figure B1B).

As subjects learned that reinforcers were always delivered to the magazine, an increase in magazine entries was gradually observed across sessions. This pattern is reflected by the significant main effect of Session on magazine entries ($F_{(5, 54.55)}=70.74$), $p<0.001$; Figure B1D). A significant Strain \times Session interaction ($F_{(1, 25.33)}=15.78$, $p<0.002$; Figure B1D) indicated a greater number of magazine entries for SD rats as compared to WKY rats during Session 1, and again on Session 6 ($F_{(1,25.23)}=5.04$, $p<0.04$; Figure B1D). Finally, there was a significant main effect of Session on magazine entries per reinforcer earned ($F_{(5,52.55)}=25.92$, $p<0.001$; Figure B1E), indicating an increase across sessions.

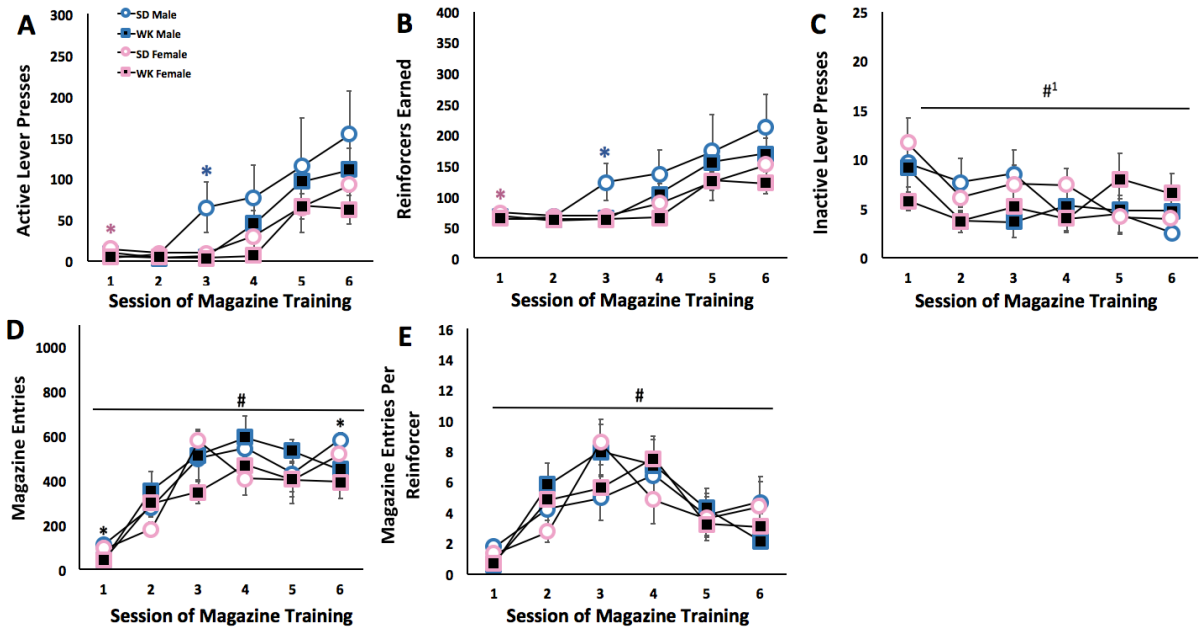


Figure B1. Magazine training performance in SD and WKY male (blue) and female (pink) rats across training sessions. Mean (\pm SEM) values across magazine training sessions for (A) active lever presses, (B) reinforcers earned, (C) inactive lever presses, (D) magazine entries, (E) magazine entries per reinforcer. * indicates a significant main effect of strain ($p < 0.05$), # indicates a significant main effect of session ($p < 0.05$), #¹ indicates a significant main effect of session for SD rats ($p < 0.05$).

B. Fixed Ratio (FR) 1 Training

Once SD and WKY rats were performing adequately in Magazine Training, all were advanced to FR1 Training. Here, sex differences began to emerge. Specifically, males tended to outperform females in terms of active lever presses and reinforcers earned.

There was a significant main effect of Session on active lever presses ($F_{(4,72.57)}=5.88, p < 0.001$; Figure B2A) indicating an overall increase in responding across sessions. There was also a significant effect of Sex ($F_{(1,27.17)}=11.50, p < 0.003$; Figure B2A) indicating that males tended to perform more active lever presses than females.

Given this pattern, it is unsurprising that there was also an overall significant effect of Session on reinforcers earned ($F_{(4,72.74)}=5.84, p < 0.001$; Figure B2B) indicating an overall

increase in sucrose pellets earned with training. The overall significant effect of Sex on reinforcers earned ($F_{(1,27.18)}=11.52, p<0.003$; Figure B2B) revealed that males tended to earn a greater number of reinforcers than females, for both strains.

As observed during Magazine Training, inactive lever pressing continued to decline across Sessions during FR1 training, as indicated by the overall significant effect of Session on inactive lever presses ($F_{(4, 57.22)}=5.16, p<0.002$; Figure B2C). A significant Strain \times Sex interaction ($F_{(1,33.82)}=6.02, p<0.02$; Figure B2C) indicated that male WKY rats tended to have greater inactive lever presses than male SD rats, while female SD rats tended to have greater inactive lever presses than male SD rats (Strain \times Sex interaction, ($F_{(1,35.21)}=9.56, p<0.005$)).

There was an overall significant effect of Sex on magazine entries ($F_{(1,29.77)}=12.35, p<0.002$; Figure 2D) indicating that males tended to enter the magazine more frequently than females. A significant Strain \times Session interaction ($F_{(1,21.19)}=15.00, p<0.002$; Figure B2D) indicated that SD rats entered the magazine more frequently than WKY rats on Session 2, while only SD rats tended to increase more frequently across sessions ($F_{(4,51.44)}=2.96, p<0.03$). Finally, there was an overall significant effect of Session on magazine entries per reinforcer earned ($F_{(4,33.70)}=4.17, p<0.009$; Figure B2E) indicating that this score decreased, overall, across sessions.

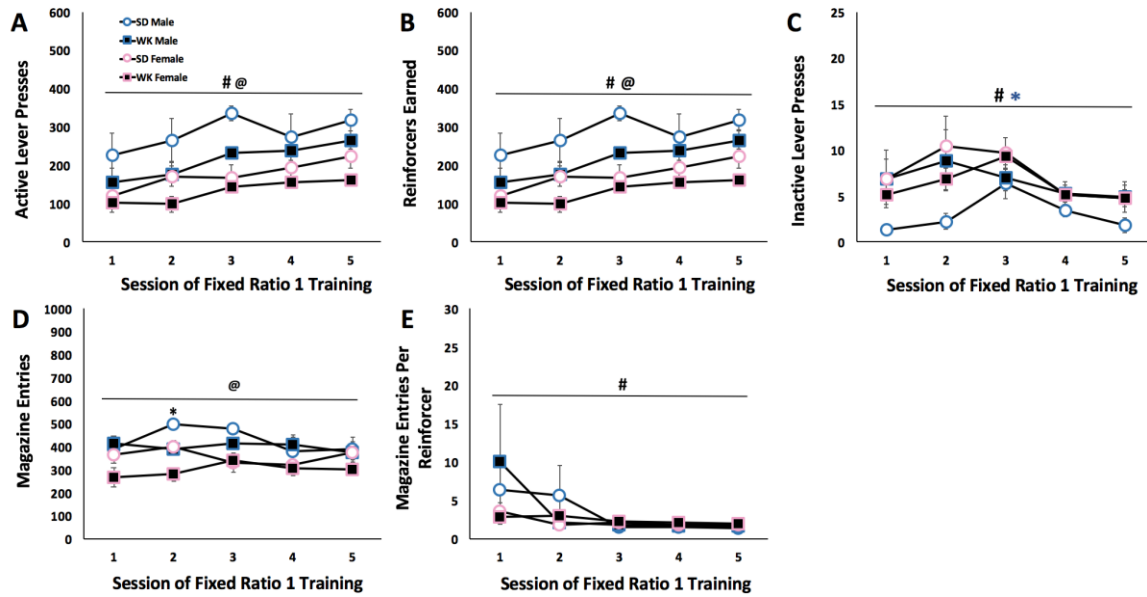


Figure B2. FR1 performance in SD and WKY male (blue) and female (pink) rats across training sessions. Mean (\pm SEM) values across FR1 training sessions for (A) active lever presses, (B) reinforcers earned, (C) inactive lever presses, (D) magazine entries, (E) magazine entries per reinforcer. * indicates a significant main effect of strain ($p < 0.05$), # indicates a significant main effect of session ($p < 0.05$), @ indicates a significant main effect of sex ($p < 0.05$).

C. Fixed Ratio (FR) 5 Training

Despite the beginning of observable differences during FR1 Training, all groups progressed to FR 5 Training. At this stage, group differences persist.

As in previous stages, male subjects continued to outperform females. There was an overall significant effect of Sex ($F_{(1, 27.97)} = 9.44, p < 0.006$) for active lever presses indicating this effect. The overall significant effect of Session ($F_{(8, 218.04)} = 37.20, p < 0.001$; Figure B3A) indicated an increase in responses across training. A significant Strain x Sex x Session interaction ($F_{(1, 41.12)} = 4.64, p < 0.04$; Figure B3A) revealed that male SD rats had increased active lever presses, as compared to male WKY rats, on Sessions 7 and 9.

Mirroring the active lever pressing results, there was an overall significant effect of Sex ($F_{(1,27.98)}=9.44, p<0.006$) for reinforcers earned, indicating that males tended to earn more reinforcers than females, for both strains. The overall significant effect of Session ($F_{(8,218.05)}=37.18, p<0.001$; Figure B3B) indicated an overall increase in reinforcers earned across training. A significant Strain x Sex x Session interaction ($F_{(1,41.18)}=4.81, p<0.04$; Figure B3B) revealed that male SD rats earned more reinforcers, as compared to male WKY rats, on Sessions 7 and 9.

As with other training stages, inactive lever presses are highest during Session 1 of FR5. As subjects begin to comprehend the new rule in place, these incorrect responses rapidly decrease. The overall significant effect of Session ($F_{(8,206.50)}=6.30, p<0.001$; Figure B3C) indicates this overall decrease across sessions. The same trend was found for magazine entries, with fewer scores overall across sessions (main effect of Session ($F_{(8,219.91)}=24.55, p<0.001$); Figure B3D).

There was an overall significant effect of Session ($F_{(8,78.00)}=20.84, p<0.001$; Figure B5E) on magazine entries per reinforcer, indicating an overall decrease across sessions. The overall significant effect of Sex ($F_{(1,30.82)}=18.17, p<0.001$; Figure B3E) indicated the tendency for females to have higher scores for magazine entries per reinforcer than males.

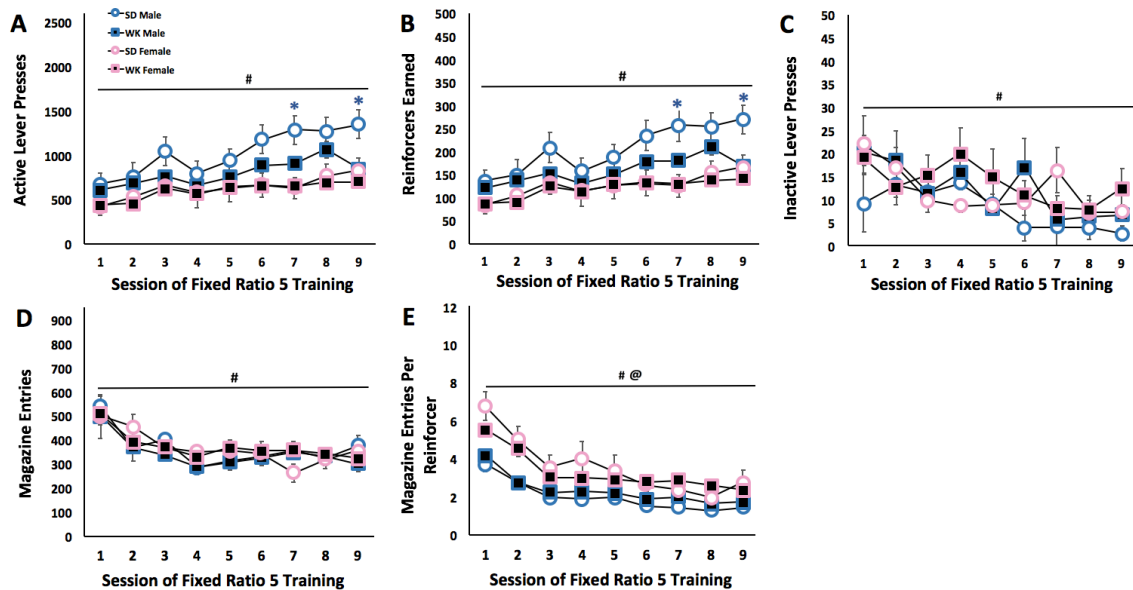


Figure B3. FR5 performance in SD and WKY rats across training sessions. Mean (\pm SEM) values across FR5 training sessions for (A) active lever presses, (B) reinforcers earned, (C) inactive lever presses, (D) magazine entries, (E) magazine entries per reinforcer. * indicates a significant main effect of strain for males ($p<0.05$), # indicates a significant main effect of session ($p<0.05$), @ indicates a significant main effect of sex ($p<0.05$).

D. Progressive Ratio (PR) Training

At the conclusion of FR5 Training, all groups have demonstrated their ability to learn new tasks and to perform well in the operant chambers, despite any group differences. However, once subjects reach the Progressive Ratio Training, strain differences start to become less apparent. Interestingly, sex differences are reversed at this point, with females beginning to outperform males.

There was an overall significant effect of Session ($F_{(9,97.24)}=8.12, p<0.001$; Figure B6A) for active lever presses, indicating an overall increase for females and overall decrease for males. The Sex x Session interaction revealed that females had greater active lever presses, compared to males, on Sessions 1, 5, 6, 7, 9 and 10 (Strain x Sex Interaction, $F_{(1, 41.12)}=4.44, p<0.05$; Figure B4A) a Sex x Session interaction effect

($F_{(9,200.13)}=5.51, p<0.001$; Figure B4B) indicated that males earned fewer reinforcers across sessions. The Sex x Session interaction revealed that females earned more reinforcers, compared to males, on Sessions 5, 6, 9 and 10 (Strain x Sex Interaction, $F_{(1,115.09)}=6.04, p<0.02$; Figure B6B)

Active lever presses also relates to the highest ratio completed, thereby producing a similar pattern by necessity. An overall significant effect of Session ($F_{(9,241.01)}=6.86, p<0.001$; Figure B4C) for highest ratio completed, indicating overall higher scores for females and lower for males. The Sex x Session interaction revealed that females reached higher ratios completed, compared to males, on Sessions 4, 5, 6, 9 and 10 (Strain x Sex Interaction, $F_{(1, 93.75)}=6.53, p<0.02$; Figure B4C).

As per usual, there was an overall significant effect of Session ($F_{(9,80.03)}=12.38, p<0.001$; Figure 4D) for inactive lever presses, indicating an overall decrease in inactive responses. The overall significant effect of Sex ($F_{(1,38.27)}=5.81, p<0.03$; Figure B4D) indicated that females tended to have increased inactive lever presses as compared to males.

There was an overall significant effect of Session ($F_{(9,90.44)}=28.64, p<0.001$; Figure B4E) for magazine entries, indicating that overall this score dropped with additional sessions. The same trend was also true for magazine entries per reinforcer (main effect of Session, ($F_{(9,75.20)}=30.68, p<0.001$); Figure B4F). The overall significant effect of Sex ($F_{(1,35.381)}=59.942, p<0.001$; Figure B4E) for magazine entries indicated that females tended to have higher scores than males for magazine entries, across sessions. This same trend was also true for magazine entries per reinforcer (main effect of Sex, ($F_{(1,38.12)}=78.43, p<0.001$); Figure B4G).

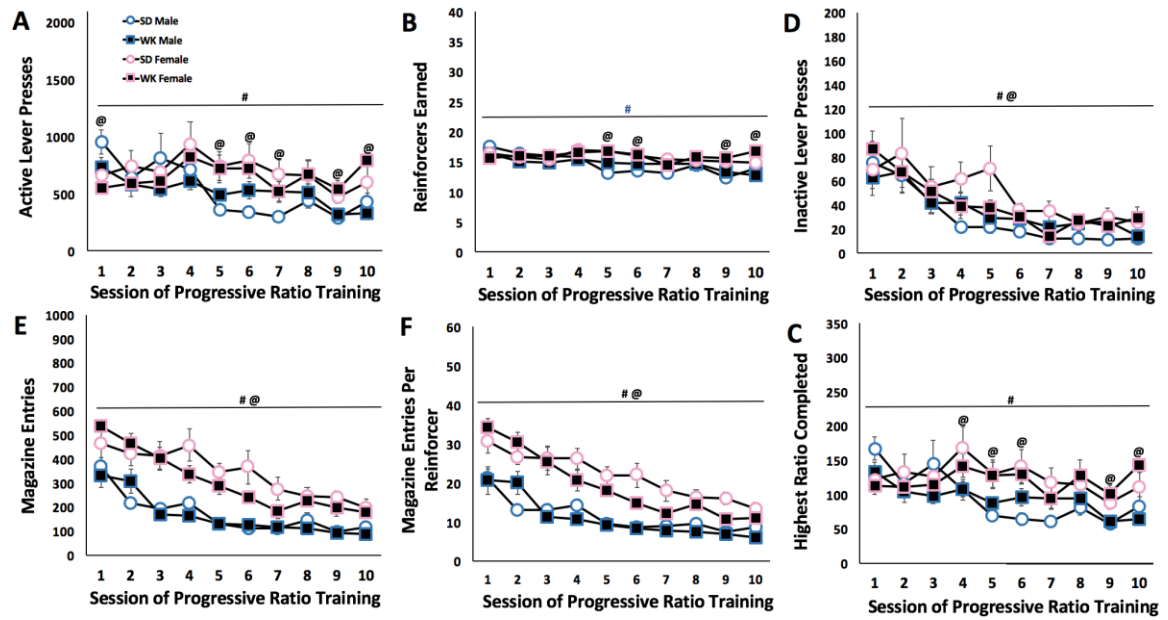


Figure B4. PR performance in SD and WKY rats across training sessions. Mean (\pm SEM) values for (A) active lever presses, (B) reinforcers earned, (C) highest ratio completed, (D) inactive lever presses (E) magazine entries, (F) magazine entries per reinforcer. # indicates a significant main effect of session ($p < 0.05$), # indicates a significant main effect of session for males ($p < 0.05$), @ indicates a significant main effect of sex ($p < 0.05$).

E. Mixed FR5-PR Training: FR5 Phase

The original pattern of sex differences persisted from FR5 Training to the FR5 Phase of mixed FR5/PR Training: male subjects tend to outperform females.

For active lever presses, male rats improved across sessions (Strain x Sex x Session Interaction ($F_{(17, 452.88)} = 2.09, p < 0.008$; Figure B5A). On Session 14, WKY males outperformed WKY females (Strain x Sex x Session interaction ($F_{(1, 40.68)} = 8.13, p < 0.008$; Figure B5A), and on Session 17 SD males had higher active lever presses than SD females ($F_{(1, 39.57)} = 4.81, p < 0.04$; Figure B5A).

This same pattern was found for reinforcers earned. A significant Strain x Sex x Session interaction ($F_{(17, 452.88)} = 2.11, p < 0.007$; Figure B5B) indicated that males earned more across Sessions. On Session 14, WKY males earned more than WKY females (Strain

x Sex x Session interaction ($F_{(1, 40.69)}=8.05, p<0.008$); Figure B5B), and on Session 17 SD males earned more than SD females ($F_{(1,39,57)}=4.79, p<0.04$); Figure B5B).

As per usual, for inactive lever presses, a significant overall effect of Session ($F_{(17, 440.93)}=3.39, p<0.001$) indicated that overall inactive lever presses decreased across sessions. A significant Sex x Session interaction ($F_{(1,30.80)}=4.72, p=0.04$) indicated that females had greater inactive lever presses on Session 15, only. A significant Strain x Session interaction ($F_{(1, 30.09)}=8.02, p<0.009$; Figure B5C) revealed that WKY rats had greater inactive lever presses than SD rats on Sessions 2 and 5, only.

While there were no significant effects of Sex, Session, or Strain for magazine entries, there was an overall significant effect of Sex ($F_{(1, 30.04)}=11.89, p<0.001$; Figure B5E) on magazine entries per reinforcer, indicating that females tended to have higher scores than males. The overall significant effect of Session ($F_{(17, 139.83)}=1.82, p<0.04$; Figure B5E) indicated that overall scores dropped across sessions.

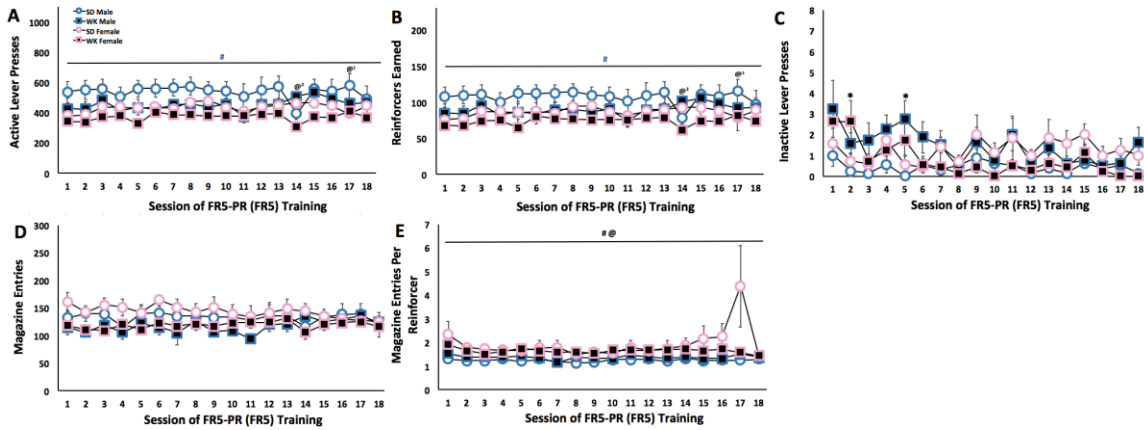


Figure B5. FR5 performance in SD and WKY male (blue) and female (pink) rats across mixed FR5/PR training sessions. Mean (\pm SEM) values for (A) active lever presses, (B) reinforcers earned, (C) inactive lever presses, (D) magazine entries, (E) magazine entries per reinforcer. * indicates a significant main effect of strain ($p < 0.05$), @ indicates a significant main effect of sex ($p < 0.05$), @¹ indicates a significant main effect of sex for SD rats ($p < 0.05$), @² indicates a significant main effect of sex for WKY rats ($p < 0.05$), # indicates a significant main effect of session ($p < 0.05$).

F. Mixed FR5/PR Training: PR Phase

Despite a lack of clear or consistent strain differences during the PR Training, these differences began to emerge during the PR Phase of the mixed FR-PR training. The sex differences of the PR phase did, however, carry over into this new stage.

There was an overall significant effect of Sex ($F_{(1,27.94)}=4.35, p < 0.05$) for active lever presses, indicating that females tended to score higher in this measure than males. The overall significant effect of Session ($F_{(17,457.04)}=2.42, p < 0.002$) reflects the WKY females and SD males improvement and the WKY males and SD females decrease in active lever presses, across sessions. Male SD rats performed more active lever presses than male WKY rats on Sessions 6 and 7 (Strain x Sex x Session Interaction, ($F_{(1,75.15)}=5.88, p < 0.02$); Figure 7A). WKY females performed more active lever presses than SD females on Sessions 6 and 13 (Strain x Sex x Session Interaction ($F_{(1,75.15)}=4.095, p =] < 0.05$); Figure B6A).

Similarly, a Strain x Sex x Session interaction demonstrated that SD males, WKY males, and WKY females were affected by session in terms of reinforcers earned ($F_{(17, 467.97)}=1.89, p<0.02$). Specifically, WKY females earned fewer reinforcers across sessions, while SD males and WKY males earned more across sessions. It was also revealed that on sessions 4, 6-13, 17, and 18, WKY females earned more reinforcers than WKY males (Strain x Sex x Session interaction, ($F_{(1, 63.55)}=4.17, p<0.05$)). The Strain x Sex x Session interaction ($F_{(1, 63.55)}=5.68, p<0.03$; Figure 7B) indicated that WKY females earned more than SD females on session 13. Meanwhile, male SDs earned more reinforcers than WKY males on sessions 6 and 7 (Strain x Sex x Session interaction, ($F_{(1, 63.55)}=4.89, p<0.04$); Figure B6B).

For highest ratio completed, there was an overall significant effect of Sex ($F_{(1, 27.94)}=4.37, p<0.05$) indicating that females tended to reach higher ratios than males. The overall significant effect of Session ($F_{(17, 460.02)}=2.34, p<0.003$) reflects the trend of WKY females and SD males improvement and the trend of WKY males and SD females decrease in highest ratio completed, across sessions. A significant Strain x Sex x Session interaction ($F_{(1, 71.65)}=4.82, p<0.04$; Figure B6C) indicated that male SD rats reached a higher ratio than male WKY rats on Sessions 6 and 7. Meanwhile, WKY females reached a higher ratio than SD females on Session 6, 13, and 14 (Strain x Sex x Session Interaction ($F_{(1, 75.83)}=4.13, p<0.05$); Figure B6C).

As observed during previous stages, inactive lever presses decreased across Sessions as subjects adapted to the new operant procedure, as indicated by the overall significant effect of Session ($F_{(17, 94.73)}=2.19, p<0.01$). A significant Sex x Strain interaction ($F_{(1, 56.15)}=22.05, p<0.001$) indicated that, for SD rats only, females had higher inactive lever

presses than males, and that female SD rats had more inactive lever presses than female WKY rats (Sex x Strain Interaction, ($F_{(1,55.76)}=27.45, p<0.001$); Figure B6D). A significant Strain x Session interaction ($F_{(1,39.35)}=5.58, p<0.03$, Figure B6D) indicated that SD rats had higher scores than WKY rats on sessions 6, 8, and 10.

For magazine entries, the overall significant effect of Sex ($F_{(1,45.35)}=51.36, p=0.000$) indicated that females tended to enter the magazine more frequently than males. For SD and WKY female group, there was a significant decrease in magazine entries across sessions (Strain x Sex x Session interaction, ($F_{(17, 126.69)}=4.28, p<0.001$); Figure B6E). On sessions 1, 3, 5, 8, 9, 10, 11, 14, and 15, SD females entered the magazine more often than SD males (Strain x Sex x Session interaction, ($F_{(1, 29.22)}=4.38, p<0.05$); Figure B6E). On sessions 5, 9, 16, and 18, SD males entered the magazine more often than WKY males (Strain x Sex x Session interaction, ($F_{(1, 29.13)}=5.07, p<0.04$); Figure B6E).

For magazine entries per reinforcer, the overall significant effect of Sex ($F_{(1,47.23)}=62.70, p<0.001$) reflected the trend for females to have higher scores than males. A Strain x Sex x Session interaction demonstrated that SD males, SD females and WKY females had lower magazine entries per reinforcer scores across sessions (Strain x Sex x Session interaction, ($F_{(17, 460.01)}=1.87, p<0.02$; Figure B6F). On sessions 1-6, 8-11, and 13-16, SD females had higher scores for magazine entries per reinforcer than WKY females (Strain x Sex x Session interaction, ($F_{(1, 30.71)}=4.45, p<0.05$; Figure B6F). On sessions 9, 11, 16, and 18, SD males had higher scores for magazine entries per reinforcer than WKY males (Strain x Sex x Session interaction, ($F_{(1, 25.2)}=4.33, p<0.05$; Figure B6F).

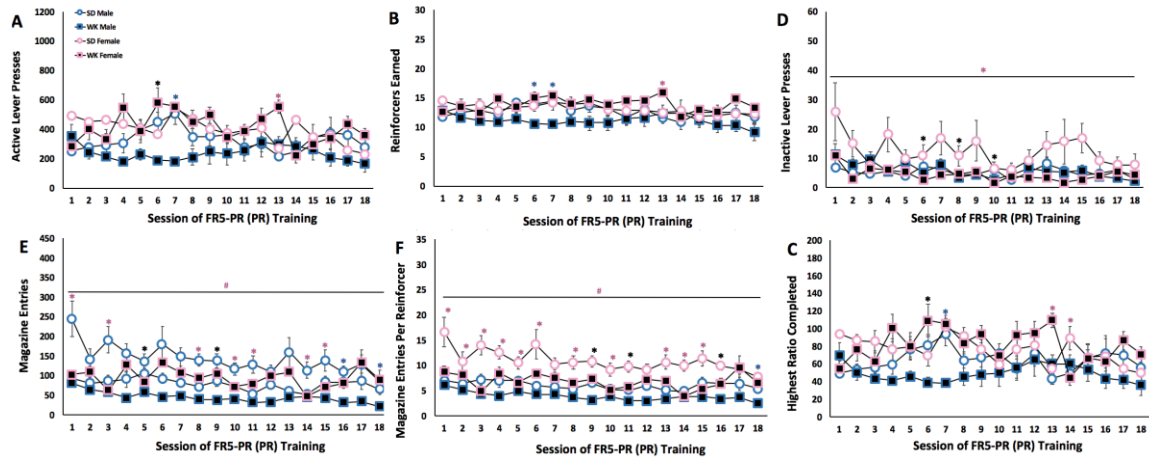


Figure B6. PR performance in SD and WKY male (blue) and female (pink) rats across mixed FR5/PR training sessions. Mean (\pm SEM) for (A) active lever presses, (B) reinforcers earned, (C) highest ratio completed, (D) inactive lever presses (E) magazine entries, (F) magazine entries per reinforcer. * indicates a significant main effect of strain ($p < 0.05$), # indicates a significant main effect of session ($p < 0.05$).

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